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ORIGINAL ARTICLE

Sublethal effects of diazinon, fenitrothion and chlorpyrifos on the functional response of predatory bug, *Andrallus spinidens* Fabricius (Hem.: Pentatomidae) in the laboratory conditions

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Abstract The sublethal effects of diazinon, fenitrothion and chlorpyrifos on the functional response of predatory bug, *Andrallus spinidens* Fabricius (Hem.: Pentatomidae), a potential biological control agent, were studied on 5th-instar nymphs. The experiment was conducted in varying densities (2, 4, 8, 16, 32 and 64) of last instars larvae of *Chilo suppressalis* Walker (Lepidoptera: Pyralidae) as prey at 25 ± 2 °C, 60% ± 10% relative humidity (RH) and a photoperiod of 16:8 h (L: D). The results of logistic regressions revealed a type II functional response in the control and all insecticide treatments. Comparison of functional response curves revealed that tested insecticides markedly decreased the mean of preys consumed by *A. spinidens*. Among them, functional response curve of *A. spinidens* in chlorpyrifos treatment was significantly lower than the other treatments. In this study, application of insecticides caused a decrease in the attack rate and an increase in the handling time of exposed bugs compared with the control. The longest handling time (3.97 ± 0.62) and the lowest attack rate (0.023 ± 0.007) were observed in chlorpyrifos and fenitrothion treatments, respectively. The results suggested that the adverse effect of these insecticides on *A. spinidens* should be considered in integrated pest management programs (IPM).

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1. Introduction

Rice is an important crop and is cultivated mainly in the north of Iran at Mazandaran, Guilan and Golestan provinces. It is attacked by very destructive pests like *Chilo suppressalis* Walker (Pyralidae), *Naranga aenescens* Moore (Noctuidae) and *Mythimna unipunctata* Haworth (Noctuidae). Among them,

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the rice striped stem borer, *C. suppressalis* is one of the most serious pests of rice. Khan et al. (1990) reported that the stem borers are major pests in all rice ecosystems. The pests attack the rice plant in different developmental stages causing symptoms like dead heart and white head (Rubia-Sanchez et al., 1997). The chemical control has been a prevalent tool for controlling these lepidopterous pests. Diazinon, fenitrothion and chlorpyrifos have been used extensively in rice fields (Ghassempour et al., 2002). More than 60% of chemical pesticides were used in Northern provinces of Iran against rice pests. In these regions, pesticides are applied 2–4 times during the rice cropping season (Noorhosseini, 2010). The extensive and repeated use of pesticides could cause serious problem such as possible toxicity in humans and animals. Further, side effects of pesticides on non-target organisms, secondary pest outbreaks, development of insecticide resistance and environmental pollution are also of concern (Talebi et al., 2011). For example, the residue of diazinon which is commonly used to control *C. suppressalis* was detected in the soil and surface-water of rice fields in the north of Iran. The studies have also shown that several useful soil microorganisms failed to grow on a medium containing diazinon (Ghassempour et al., 2002). In the rice ecosystem, natural enemies include predators and parasitoids, which are considered as important biological agents for controlling various insect pests. Conservation of natural enemies in the rice fields may suppress the pest populations, which in turn will reduce the rate of insecticide application (Jadhao, 2011).

Andrallus spinidens Fabricius is a non-specific predator on lepidopterous larvae in rice fields (Manley, 1982). Second to fifth instar nymphs and adults of *A. spinidens* have predatory activity on caterpillar pests of rice like *C. suppressalis*, *N. aenescens* and *M. unipunctata* (Nageswara Rao, 1965; Manley, 1982; Mohaghegh and Najafi, 2003; Behera and Prakash, 2004). This pentatomid bug has a critical role in the regulation of rice pest's population (Najafi-Navaee et al., 1998). There are three factors which should favor *A. spinidens* as a potentially useful biological control agent of rice pests: relatively short life cycle, aggressive feeding behavior and ability to feed continually for several hours (Manley, 1982). This natural enemy may be affected by insecticide sprays in rice fields via direct contact with residues, or indirectly through contaminated food. Integrating the application of biocontrol agents and insecticides for Integrated Pest Management (IPM) in rice ecosystem requires knowledge about impact and selectivity of the insecticides on natural enemies (Croft, 1990; Dent, 1995).

The control of a pest by a predator depends strongly on the predator–prey interaction such as the predator's numerical and functional responses (Holling, 1959). Functional response tests show the potential of parasitoid/predator ability to suppress the different densities of prey/host. In fact, it describes the way a natural enemy responds to the changing densities of its prey and it is a commonly measured attribute of natural enemies. Holling (1959, 1966) proposed three types of functional responses. In type I, number of killed host/prey rises as linear to a plateau; type II, a curvilinear rise to a plateau which then levels off under the influence of handling time or satiation and in type III predator/parasitoid response by a sigmoid increase in prey/hosts attacked (Hassell, 2000; Mills and Lacan, 2004). Many factors such as pesticides influence the functional response of a predator (Murdoch and Oaten, 1975). Several studies provided a strong evidence that insecticides affect the functional response of natural enemies (e.g. Gu, 1991; Jebanesan, 1998; Wang and Shen, 2002; Claver et al., 2003; Deng et al., 2007; Ambrose et al., 2008, 2010; Rafiee-Dastjerdi et al., 2009; Rezac et al., 2010; Abedi et al., 2012). The functional response of *A. spinidens* to different densities of larvae of *N. aenescens* has been studied by Javadi and Sahragard (2005). However, there is no data about the investigation of insecticides on functional response of the predatory bug. In this study, we examined the sublethal effects of three insecticides, diazinon, fenitrothion and chlorpyrifos on the functional response of *A. spinidens*. Such information can be used to predict the potential of these pesticides in combination with *A. spinidens* in controlling rice pests.

2. Materials and methods

2.1. Insect rearing

The adults and nymphs of *A. spinidens* were collected from rice fields in Amol, Mazandaran province (north of Iran), in late September 2012. These insects were reared on last larval instar of *Galleria melonella* Linnaeus (Lep.: Pyralidae) in the laboratory conditions (25 ± 2 °C, 60% ± 10% RH and a photoperiod of 16:8 h L: D) h. The second generation of *A. spinidens* was used for experiments. Also, pupae of *C. suppressalis* were collected from the rice field in late may 2012 and kept in rearing chamber as above. After the emergence of adults and subsequent copulation and egg laying, the hatched larvae were reared on the rice seedling *Oryza sativa* L. (Taroum variety). The last larval instar of *C. suppressalis* was used as prey for functional response experiment of *A. spinidens*.

2.2. Pesticides

The pesticides used in this study were technical material of diazinon (Gyah Corporation, Iran, 99.8% purity), fenitrothion (Pesticides and Agriculture Research Center, Iran, 99.8% purity) and chlorpyrifos (ACO, USA, 99.9% purity).

2.3. Bioassay

Initially, preliminary bioassays were conducted to determine the effective concentrations caused between 10% and 90% mortality. The insecticides were bioassayed at serial concentrations in ranges of 1000–3500, 200–800 and 200–950 ppm a.i., for diazinon, fenitrothion and chlorpyrifos, respectively. These insecticides were diluted in acetone (Merck Company, Germany) and 1 μl of each concentration was applied topically using a microapplicator on the thoracic dorsum of newly molted 5th-instar nymphs of *A. spinidens*. Control treatment received 1 μl of acetone alone. Forty nymphs of *A. spinidens* were used for each concentration and the control. Mortality was assessed 24 h after treatment and the LC_{30} value of each insecticide was estimated.

2.4. Functional response assay

In order to evaluate the searching efficiency of *A. spinidens*, the functional response of this predator to different densities of *C. suppressalis* was studied. Twenty-four hours after treatment